ASSIMILATION OF DAIRY WASTES BY ACTIVATED SLUDGE

By Sam R. Hoover, Lenore Jasewicz, Janet B. Pepinsky, and Nandor Porges

Eastern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Dept. of Agriculture, Philadelphia, Pa.

Studies of the aerobic oxidation of a simulated dairy waste (0.10 per cent skim milk solids) in a continuous flow system showed that one-half the soluble solids was rapidly converted to cell substance and the remainder was oxidized completely to carbon dioxide and water (6). These results were obtained by determination of the amount and composition of milk waste introduced and of the resulting soluble products and insoluble cell residue. This paper reports a manometric study of the oxidation of skim milk and its major components, casein and lactose, by a mixed culture from an activated sludge plant successfully treating the waste from a small market milk plant.

Application of manometric methods in the study of waste disposal problems, especially the determination of B.O.D., has been proposed by various workers. Wooldridge and Standfast (14)(15)(16) made an excellent study of the effect of temperature, pH, and supersaturation of sludge with sewage on the rate of oxidation. These workers also established that carbon dioxide is the only gas produced in this oxidation; an essential condition for the use of the simple manometric procedures. Caldwell and Langelier (1) analyzed thoroughly the theory and practice of the various manometric techniques and proposed an adaptation of the Warburg apparatus in which the usual 15-ml. vessels are replaced by 125-ml. flasks. They recommend its use in routine determinations of B.O.D. of trade wastes and in sewage research studies. Dawson and Jenkins (3) recently reported an extensive study of oxygen requirements by activated sludge in which the effects of a number of variables were measured. These papers should be consulted by workers interested in the application of manometric methods to a broad range of sewage problems.

The techniques required in the study of bacterial and tissue respiration have been well worked out, and the standard Warburg apparatus is especially well suited for such research. These procedures have been applied just as they are described in standard published methods (4)(13).

Experimental

Six liters of activated sludge were placed in the 20-l. Humfeld aerator. This culture was developed to 20 l. by adding a solution containing 0.1 per cent skim milk solids at a rate of 1.1 l. per hr., and was maintained at that volume by feeding continuously at this rate and removing the effluent. The temperature was 30° C. Other details of the procedure have been described previously (6). Manometric experiments were initiated on the second day and completed within three weeks. A later series of experiments was conducted on sludge within an hour of the time it was obtained from the plant. Control experiments showed that the culture in the aerator retained its oxidative efficiency for several hours

without feeding and that the soluble nutrients were low. Therefore, the culture was not centrifuged and resuspended in the medium, but was used directly in the Warburg vessels with substrate added from the side arm. In about an hour, the time required to set up the experiment, the residual nutrients were almost completely oxidized. Oxygen demand was determined in the solutions by chemical oxygen demand determination. The chemical oxygen demand has been shown to be essentially equal to the 20-day B.O.D. on skim milk solids (11). In all experiments, nitrogen was supplied in excess as ammonium sulfate to compensate for its absence in lactose.

Figure 1 shows the rate and amount of oxidation of skim milk and of its two principal components in the approximate proportion in which they occur in skim milk. This experiment was performed on the second day after the culture was obtained. The similar rate and extent of oxidation of 0.50 mg. lactose per ml. of culture and 0.35 mg. of casein per ml. is a result obtained repeatedly. The culture without added nutrients had a low and practically constant endogenous respiration, oxidizing cell constituents for maintenance energy. The rate of oxidation in the vessels containing nutrients was high for a few hours, but dropped to almost the rate of the control in about 6 hr.

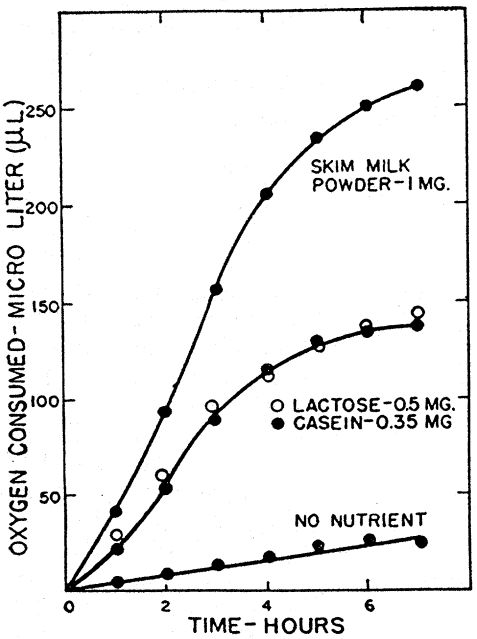

FIGURE 1.—Rate of oxidation of skim milk, lactose, and casein by 1.0 ml. of sludge cultured in fermentor for 2 days.

TABLE I.—Oxygen Utilization by Various Nutrients

Nutrient		Oxygen Utilization		
Type	Mg.	Theory (μL.)	Observed[1] (μL.)	%
Lactose	0.5	371	136	37
Casein	0.35	335	133	40
Skim milk powder	1.0	780	250	32

The percentage of the oxygen theoretically * required for complete oxidation was calculated from these data (Table I). Oxidation of only 32 to 40 per cent for the three substrates can be taken as indicating either partial oxidation or assimilation (growth of the cells). Because previous results showed there were negligible quantities of oxidizable substances left in the medium,

Theoretical factors for complete oxidation of nutrients were calculated as follows:

Lactose:—Complete oxidation of one mole requires 12 moles O_2, or 1.06 g. O_2 per gram of lactose hydrate, 1.06 μg. O_2 = 0.742 μL. O_2. 0.5 mg. or 500 μg. = 371 μL. O_2.

Casein:—Analysis of this sample gave: C = 53.0 per cent; H = 7.0 per cent; N = 15.7 per cent; and O = 27.7 per cent. Complete combustion of carbon and hydrogen, assuming N is converted to NH_3, requires 1.48 g. O_2 per gram of casein, or 1.035 μL. O_2 per μg. casein. 350 μg. air dry casein (8 per cent moisture) = 335 μL. O_2.

Skim milk:—The sample used contained 36.3 per cent protein and 50.0 per cent lactose anhydride. The remainder is primarily ash and water. A calculation similar to the one above gives the value of 0.780 μL. O_2 per μg. air-dried skim milk. 1.0 mg. or 1,000 μg. = 780 μL. O_2.

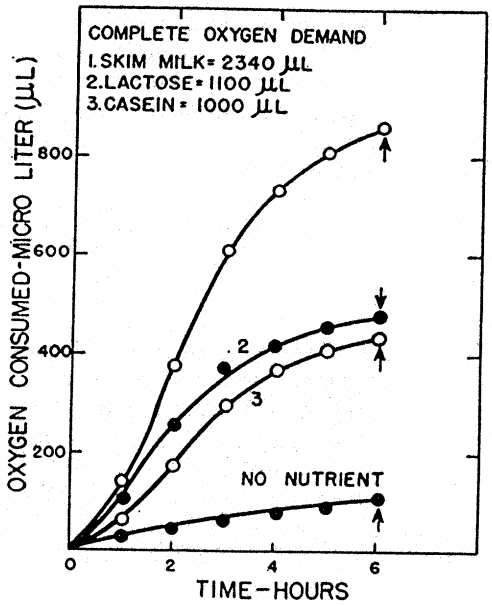

FIGURE 2.—Rate and extent of oxidation of skim milk, lactose, and casein by 3 ml. of sludge cultured in fermentor for 7 days. Arrows indicate when samples were taken and analyzed for residual substrate.

the second possibility was investigated.

A similar experiment (Figure 2) was therefore run in which the 1 ml. of culture per vessel was increased to 3 ml., with an accompanying 3-fold increase in added nutrients. After 6 hr., the measurements were stopped, and the contents of the vessel chilled and centrifuged cold. Residual substrate and/or partial oxidation products in the supernatant were determined by chromic acid oxidation (chemical oxygen demand) (11). The oxygen demand was 92 p.p.m. in vessel 1, 60 p.p.m. in vessel 2, 96 p.p.m. in vessel 3, and 72 p.p.m. in vessel 4. The culture put into the vessels originally contained 118 p.p.m. chemical oxygen demand. The added nutrients, therefore, had been completely removed from the solution. The calculated oxidation was 43 per cent for lactose and casein, and 37 per cent for skim milk, slightly higher than in the previous experiment. These results can only be interpreted as evidence of assimilation of the remaining 57 to 63 per cent of the protein and carbohydrate into cell tissue, an assimilation slightly higher than the 50 per cent obtained in the continuous flow experiments. The energy for this growth process was obtained from the portion of the substrate oxidized.

Respiratory quotient (R.Q. = CO_2/O_2) determinations made on the system further confirm the idea that the substrate is assimilated or oxidized completely. The results given in Table II are preliminary, but they correspond to a complete oxidation to CO_2 and water, with an R.Q. of slightly higher than 1.0 for lactose and skim milk and close to 1.0 for casein. The theoretical R.Q. for complete oxidation of lactose is 1.00; for casein it is 0.96 if the oxidation of carbon and hydrogen is complete and nitrogen is assumed to be produced as ammonia. The synthesis of cell tissue would be expected to have an R.Q. slightly greater than these

TABLE II.—Rate of Oxidation and Respiratory Quotient of Activated Sludge Culture Obtained May 31, 1950, and Maintained Aerobically in the Laboratory

Date	Oxidation Quotient (µL./mg./hr.)[1]				Respiratory Quotient (CO_2/O_2)[1]		
	None	Casein	Lactose	Skim Milk	Casein	Lactose	Skim Milk
6- 2-50	7	62	60	106	—	—	—
6- 7-50	18	84	96	168	—	—	—
6-20-50	—	116	90	183	1.00	1.03	1.03
6-21-50	—	—	114	—	—	1.06	—
6-22-50	—	111	96	—	1.00	1.03	—

theoretical values, for the cell tissue which is synthesized has less oxygen in it than the substrates. The difference of about 0.05 is in agreement with this expectation.

The data on rate of oxidation are given in Table II as the oxidation quotient, which is the μL. O_2 consumed per mg. per hr. It is an absolute measure of the rate of oxidation of substrate by a culture.

The culture under aerobic conditions in the laboratory became more active, as the oxidation quotient shows. The relative rate of oxidation of lactose, protein, and skim milk remained essentially the same, despite the increase in activity of the culture. These results indicated that the culture studied in the laboratory was comparable in physiological properties to the activated sludge in the waste disposal plant. A specific experiment was run to confirm this indication. Fresh sludge was obtained from the plant, and the experiment was repeated on it. The results are given in Figure 3. It

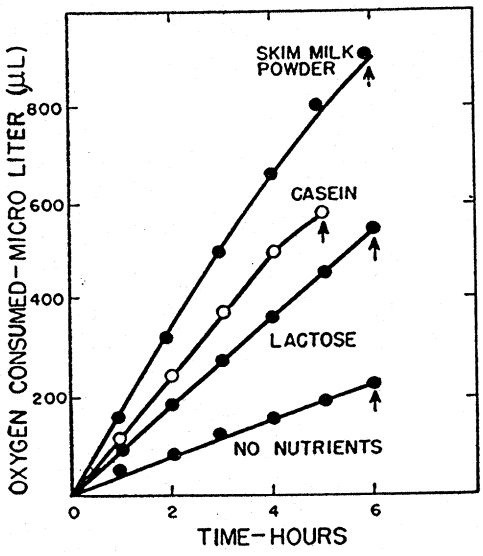

FIGURE 3.—Rate and extent of oxidation of skim milk, lactose, and casein by fresh sludge. Arrows indicate when samples were taken and analyzed for residual substrate.

can be seen that the two sets of data (Figures 2 and 3) are similar. The soluble portion of the fresh sludge contained 460 p.p.m. chemical oxygen demand. Therefore, the vessels containing no added nutrients had considerable available substrate. The oxidation quotient values were:

No added nutrients	12 μL./mg./hr.
Lactose	27 μL./mg./hr.
Casein	37 μL./mg./hr.
Skim milk	49 μL./mg./hr.

An analysis for residual chemical oxygen demand was performed after 6 hr., as indicated by the arrows in Figure 3. The lactose and casein had been completely removed and more than 90 per cent of the skim milk solids had been oxidized. The oxidation, therefore, accounted for about 50 per cent of the casein and lactose and 45 per cent of the skim milk solids. Conversely, assimilation of 50 and 55 per cent of the substrates into cell tissue is indicated.

The activated sludge contained a total chemical oxygen demand of 1,875 p.p.m., of which 1,415 p.p.m. were due to suspended cells. A chemical oxygen demand value can be converted to dry weight by use of the appropriate factor; for bacterial cells, an empirical factor of 1.25 g. O_2 per gram of dry weight was found. The sludge, therefore, contained 1,130 p.p.m. cells, a much higher value than that of about 400 to 500 p.p.m. maintained in the aerator by feeding 1,000 p.p.m. milk solids into it continuously. The data on rate of oxidation of skim milk solids on both a weight basis (oxidation quotient) and a volume basis (μL. per ml. culture per hr.), were plotted (Figure 4); the more recent results were used for the zero-day points and the data of Table II for the 2-, 7-, and 20-day points. Although there was almost a four-fold increase in the rate of oxidation on a weight basis, the culture maintained under the conditions of the experiment became more dilute, and

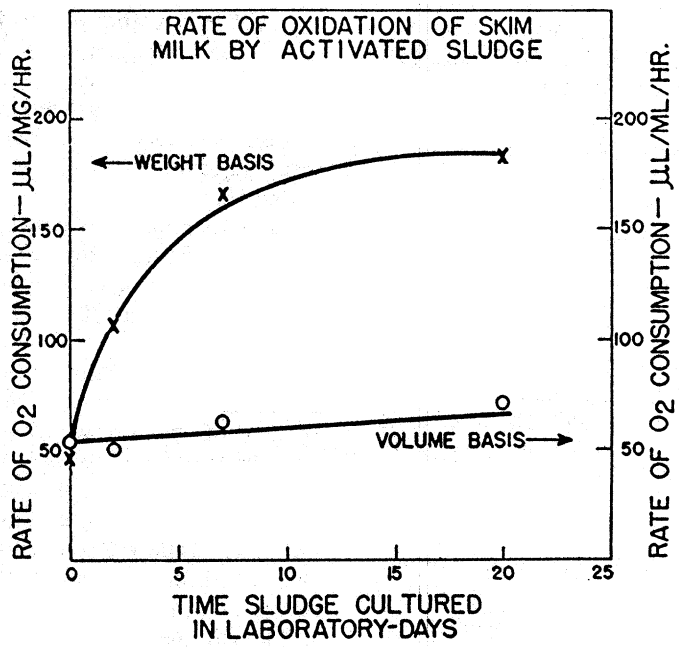

FIGURE 4.—Rate of oxidation of skim milk solids by activated sludge. Left ordinate calculated on weight of microbial cells present; right ordinate calculated on volume of culture present.

the activity per milliliter, or per cubic foot, remained essentially constant.

Discussion

The data presented in this paper are significant from two standpoints. First, the manometric determination of the amount of oxygen consumed, combined with the evidence from chemical oxygen demand determinations on the solution, proves that 60 per cent of the organic matter is assimilated into cell tissue and the remaining 40 per cent is burned to carbon dioxide and water to obtain energy for this assimilation. Second, they illustrate the type of information that can be obtained by manometric determinations in waste disposal studies. The absolute rate and extent of oxidation, the respiratory quotient, utilization of various oxidizable substances, requirements for nitrogen, potassium, and other elements, effects of pH and toxic compounds on the microorganisms, and other biochemical characteristics of the process can readily be measured. Further use of manometric methods in waste disposal studies is clearly indicated.

The efficiency of assimilation of about 60 per cent for the mixed culture studied is comparable with previous observations with bacteria and yeasts. The results are in general agreement with results of previous studies of the growth of *Rhizobium trifolii,* a symbiotic legume nodule bacterium, which grows readily on synthetic media if fixed nitrogen is supplied. An efficiency of 51.4 per cent was calculated from analysis of the respiratory quotient; determined values approached this value closely (5).

An oxidative assimilation of two-thirds of the carbon of carbohydrates and an oxidation of one-third to CO_2 has been observed in short-time experiments with various microorganisms. In 1946, Clifton (2) reviewed the subject of microbial assimilation.

Utilization of organic substrates by

activated sludge was the subject of an extensive study, which was summarized by Placak and Ruchhoft (10). B.O.D. and suspended solids determinations were used to study the assimilation of carbohydrates, proteins, organic acids, amino acids, and other materials by activated sludge in 24 hr., with the following results:

Class	Per Cent Oxidized		Per Cent Converted to Protoplasm (Organized Sludge)
	Range	Mean	
Carbohydrates	5 to 25	13	65 to 85
Alcohol	24 to 38	30	52 to 66
Amino acids	22 to 58	42	32 to 68
Organic acids	30 to 80	50	10 to 60

Peptone, which should be comparable with casein, was oxidized with an oxygen consumption of about 50 per cent of the theoretical value for complete oxidation; an accompanying increase in suspended solids accounted for the other 50 per cent. Assimilation of 80 per cent of the lactose fed is indicated. Their data on other carbohydrates indicate that a nonoxidative assimilation or true storage of carbohydrate occurred, because starch, for example, was removed from the solution without an increase in oxygen consumption. No nitrogen was supplied in these experiments, and it is probable that oxidative assimilation of the carbohydrates was prevented by lack of nitrogen.

In the present experiments, adequate nutrients were supplied for growth, and, consequently, growth or oxidative assimilation was the primary process. Otherwise the present results and those of the U. S. Public Health group (10) are in excellent agreement.

The present results are also in agreement with waste disposal studies of Jenkins and Wilkinson (7). When sufficient nitrogen was supplied, lactose was assimilated by activated sludge, with an increase in weight equal to 43 per cent of the added lactose. Lactose removal was rapid under these conditions. If nitrogen was limited, the rate of lactose removal was comparatively slow, but assimilation of the lactose produced an increase in sludge solids equivalent to 39 per cent of the added lactose.

In experimental aeration studies, Trebler (12) has repeatedly obtained B.O.D. reductions of about 50 per cent, and McKee (9) has recently reported a removal of 37 per cent in a dairy waste aeration plant utilizing no sludge separation or removal. The results of all these investigations of various types are consistent. They indicate a rapid assimilation of dairy waste by activated sludge; about 50 per cent of the waste is oxidized so that the remaining 50 per cent can be assimilated into cell tissue.

The rate of this oxidation is of great importance in plant practice. Johnson (8), for example, reported a complete absence of dissolved oxygen during the period when dairy waste containing 500 to 1,000 p.p.m. B.O.D. was being fed rapidly into an aeration tank. Any continued period of anaerobic conditions can be expected to result in acid production, with a consequent fall in pH and lessened activity of the sludge. This situation would be especially serious in a municipal sewage plant designed for the more dilute domestic waste.

Summary

The rate and extent of oxidation of skim milk, casein, and lactose by activated sludge were determined by manometric and chemical analysis. Activated sludge was successfully cultured in the laboratory without alteration of its physiological characteristics. Skim milk at a concentration of 1,000 p.p.m. was completely removed from the solution in 6 hr.; 60 per cent was assimilated into cell tissue and 40 per cent was oxidized completely to carbon di-

oxide and water. Casein and lactose were converted into cell tissue in a similar manner, but at a slightly lower rate. The results are compared with the results of previous studies of bacterial growth processes and of the metabolism of activated sludge.

Acknowledgment

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